Biometry of Seven Natural Populations of the Platyfish, *Xiphophorus maculatus*, from Central America

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(Text-figures 1-15)

The Central American platyfish, Xiphophorus maculatus (Guenther), a small viviparous cyprinodont, is found in each of the seven major Atlantic coastal river systems from southern Mexico to British Honduras. The northernmost limit of its range is the Río Jamapa near the city of Veracruz, and its southernmost habitat is in the Belize River.

The seven populations of the platyfish, a freshwater species, are geographically isolated. Their migration along the Atlantic coast is not now possible, for the species does not tolerate sea water. Owing to the relative smallness of the species-specimens rarely reach two inches under natural conditions-there is considerable difficulty in obtaining accurate measurements for the purpose of comparing one group with another. Nevertheless, the present paper is concerned with an attempt to use some of the standard methods in distinguishing the members of seven geographically isolated river populations on the basis of three body measurements and proportions: length and depth of body and length of caudal peduncle. Other measurements of the body proper were made but found not to be useful. In a later study, the analysis of the data on the frequencies of number of dorsal fin rays in similar platyfish populations will be presented.

It has been found by the authors, Gordon (1947) and Gordon & Gordon (1950, 1954), that the seven populations of the highly polymorphic platyfish are distinguishable on the basis of the gene frequencies for twelve heritable

melanic patterns. These twelve black markings are produced by two genetically different kinds of melanophores, one small, the other large. Seven black patterns, on and near the tail, produced by micromelanophores, are controlled genetically by a series of seven dominant autosomal multiple alleles. Five additional spotted patterns on the body produced by macromelanophores are controlled by a series of five dominant sex-linked multiple alleles. In each series, the unmarked platyfish is the universal recessive. On the basis of the frequencies of the twelve dominant patterns and their universal recessive, the authors (1954) worked out a "taxonomic key" to identify each of the river races, providing at least 200 platyfish in a population are available.

Other genetical analyses of the Central American platyfish and related species and their hybrids by Gordon (1948, 1950, 1951a, b) have revealed that the macromelanophore genes are potentially injurious. For example, in fish with certain genetic recombinations, the large black pigment cells grow atypically. This leads to the development of a pathological state of melanosis or of melanoma. Since about 20% of all feral platyfish have at least one large black pigment cell pattern, it was thought desirable to determine if there exist any correlations between the various macromelanophore patterns and size and body proportions.

MATERIAL AND METHODS

The northernmost population of the platyfish, *Xiphophorus maculatus*, was taken near the mouth of the Río Jamapa, in the central part of the state of Veracruz, just south of the city of Veracruz (see Text-fig. 1). To the south, another population was found in the Río Papaloapan, about 100 miles inland along the border of the state of Oaxaca. The third population was taken from various localities in the Río Coatzacoalcos

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TEXT-FIG. 1. Part of Mexico, Guatemala and British Honduras showing the seven river systems in which *Xiphophorus maculatus* were taken. The platyfish found in the Río Jamapa were taken within 10 miles of the mouth. The platyfish from the Río Grijalva were found within 20 miles of the mouth. The localities at which platyfish were taken in the other five river systems are shown in subsequent figures. The arrow above the Belize River indicates the location of two creeks of independent drainage from which platyfish were taken; the locations of these and other stations in British Honduras are shown in Text-fig. 5. This map is modified from that of Hoy, 1943.

in southern Veracruz. A fourth group was located in a small stream, Arroyo de la Venta, that entered the Río Tonalá near its mouth at the border of the states of Veracruz and Tabasco. Another large population was collected in the lowland area of the Río Grijalva near the capital of Tabasco, Villa Hermosa. The sixth major platyfish group was taken from the Lake Petén area and other parts of the upper Río Usumacinta in Guatemala. Small numbers of the species were found in the region of the mouth of the Río Hondo, the river that separates Mexico from British Honduras, but a large representative collection (the seventh) was taken from a tributary near the mouth of the Belize River just north of Belize.

The platyfish taken in the field were preserved in 10% formalin. In the laboratory they were washed in water and transferred to and stored in 70% alcohol. The specimens showed little or no shrinkage and their melanic patterns remained intact. Although nearly 9,000 platyfishes were collected, only 2,993 were measured for this analysis, of which 2,517 were males and 476 were females. The collections utilized are listed in Table 1 and their localities are shown in Textfigs. 1, 2, 3, 4 and 5. A summary of some genetic differences between the seven platyfish populations is presented diagrammatically in Text-fig. 6.

Three measurements were made, using dividers and a millimeter scale, and were recorded in millimeters: (1) *Standard Length*, the distance from the tip of the snout to the end of the vertebral column; (2) *Depth*, the distance from the base of the anterior margin of the dorsal fin to the midpoint between the origins of the pelvic fins; and (3) *Length of Caudal Peduncle*, the

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River	Collectors	Year	Field Numbers	Number of Males Measured	Depository	Location Shown in Text-fig.
Jamapa	Myron Gordon, Evelyn Gordon, James W. Atz	1939	45a, b	346	UMMZ1	1
Papaloapan	Same	1939	43a, b, c, d 38a, b, c	1331	UMMZ	2
Coatzacoalcos	Myron Gordon, James W. Atz, F. G. Wood, Jr.	1948	GAW 35, 17, 2 32, 31	3, 485	NYZS ²	3
Tonalá	Same	1948	GAW 24, 25, 2 28, 29, 30	7, 81	NYZS	3
Grijalva	Myron Gordon, Jesus Garcia	1952	GG 12	50	NYZS	1
Usumacinta	Carl L. Hubbs, Henry van der Schalie, Josslyn Van Tyne	1935	M35-8 to M35-135	176	UMMZ	4
Belize	Myron Gordon, Gerald Fairweather	1949	GF 5	48	NYZS	5
			Tot	al 2517		

TABLE 1. COLLECTIONS OF Xiphophorus maculatus UTILIZED IN THIS STUDY

¹ UMMZ–University of Michigan, Museum of Zoology.

² NYZS-New York Zoological Society.

distance from the base of the anterior margin of the anal fin to the end of the vertebral column (Text-fig. 7). In platyfish from the Río Tonalá samples, the caudal peduncle was measured from the midpoint between the origins of the pelvic fins to the end of the vertebral column instead of in the above manner. In order to compare these populations with the others properly, the measurements had to be made to correspond. A sliding scale was therefore constructed, giving the difference in the two measurements of caudal peduncle length as a function of the length of the fish. Since the correlation between standard length and the difference between the two measurements is large ($z = .96 \pm .12$), the results obtained are only slightly inaccurate. The orig-



TEXT-FIG. 2. The collection stations in the vicinity of Papaloapan, Oaxaca, at which *Xiphophorus* maculatus were taken. The numbers on the map are the original numbers of the 1939 collection except that 32-7 indicates the station at which a collection was made in 1932. Platyfish were taken at each station except station 42.



TEXT-FIG. 3. Map of the Río Coatzacoalcos and Río Tonalá systems showing the stations at which *Xiphophorus maculatus* were taken. The numbers on the map are the original numbers of the 1948 collection with the prefix GAW omitted. Platyfish were not taken at some of the stations shown.

inal measurements are used in comparison within the Río Coatzacoalcos system, but the corrected measurements are used in the comparisons between river populations.

Two form indices were calculated from these data: the *depth index*, equal to the standard length divided by the depth, and the *length of caudal peduncle index*, equal to the standard length divided by the length of the caudal peduncle.

Means, standard deviations and standard errors of means were calculated for these data. A modified formula of Simpson & Roe (1939) for the standard error of the difference between two means was used in the calculation of the critical ratio for determination of the significance of this difference:

$$\frac{\mathrm{d}}{\sigma_{\mathrm{d}}} = \frac{\mathrm{M}_{1} - \mathrm{M}_{2}}{\sqrt{\frac{\sigma_{1}^{2}}{\mathrm{N}_{2}} + \frac{\sigma_{2}^{2}}{\mathrm{N}_{1}}}}$$

The criterion of significance was taken as

 $d/\sigma_d = 2$. It should be noted that use of the above formula determines whether the observed difference in means is consistent with the hypothesis that the samples came from populations with the same mean and variance.

As an aid to visualizing the result of these comparisons, the data were graphed by a modification of the method described by Dice & Leraas (1936). In these graphs, the long vertical line represents the range of observations; the center line of the rectangle represents the arithmetic mean, and the upper and lower limits of the rectangle represent the mean plus and minus twice its standard error. A significant difference should be indicated by non-overlapping of rectangles; this is not always the case, however, because of varying sample sizes, and hence no precise conclusions can be based on the graphs. Sample sizes, given in the tables, have sometimes been omitted from the graphs for the sake of clarity.

An attempt was made to adapt the method

TEXT-FIG. 4. The collection stations in the Guatemala portion of the Río Usumacinta system at which Xiphophorus maculatus were taken. The numbers on the map indicate stations where platyfish were taken. The Xs indicate stations where Xiphophorus helleri were taken. This map is modified from that prepared by Hubbs & van der Schalie, 1937. (Reprinted from Gordon, 1947).



described by Parr (1949) for use in comparing the various populations, by taking advantage of the correlation between standard length and depth index (see below). Because of the difficulty in evaluating the significance of the differences between regression coefficients, and because the results seemed more contradictory and less accurate than those derived by the earlier procedure, the method was not used.

The question of whether there is any sexual dimorphism in the proportions of the body, aside from being of interest in itself, is important in determining whether data on both sexes may be combined in making interpopulation comparisons.

It was found impractical to dissect or section the many specimens to determine their sex. It was decided therefore, that any fish, whether completely mature or not, which showed a conspicuous andromorphic modification of the anal fin was to be classified as a male. The remaining fish were classified as female or immature, according to size and female-like body contours. On the basis of some preliminary tests, it was found that for comparative purposes a measurement of an adequate number of males was superior to a measurement of females and immature fish. As a consequence, comparisons between local populations within the Río Papaloapan, Río Coatzacoalcos and Río Usumacinta systems, as well as comparisons between each of the seven river populations taken as wholes, were made only between males. In the Río Grijalva and Belize River collections, only about 50 males were measured. The other five populations had already been analyzed, and the results indicated that no advantage, in interpopulation comparisons, would be gained by measuring more than fifty specimens from any one population.

To determine the relationship of macromelanophore pattern to size and body proportions, the members of the Río Jamapa population were classified on the basis of appropriate criteria, Tables 2, 3. Although platyfish have five macromelanophore patterns, only three of them are found in the Río Jamapa population



TEXT-FIG. 5. Map of British Honduras. The representative collection of *Xiphophorus maculatus* was taken just north of the city of Belize. Smaller collections were taken just north of Corozal, at San Antonio, at Hill Bank and at San Estevan.

(Text-fig. 6). These three are Spot-sided, Sp; Spotted-dorsal, Sd; and Stripe-sided, Sr. The absence of a pattern is designated by the plus sign, "+", the universal recessive. On the basis of the results obtained from the analysis of the Río Jamapa platyfish, it was decided that it was not advantageous to carry out this type of analysis for the platyfish of the remaining six populations.

ANALYSIS OF DATA Sexual Dimorphism

The mean standard length, depth index and length of caudal peduncle index, together with the standard errors of these means and the corresponding standard deviations, for the Río Jamapa males, are given in Table 2, and for the Río Jamapa females in Table 3. The ratio of the difference in mean standard length between



TEXT-FIG. 6. The macromelanophore patterns and their relative gene frequencies in seven river populations of *Xiphophorus maculatus*. The area of the circle for each population and pattern is proportional to the gene frequency in that population of the allele producing that pattern. For convenience in printing, the circle for *Sb* is drawn about four times its proper area. Missing circles indicate zero gene frequencies. Each of the seven river populations has a distinct set of macromelanophore gene frequencies. This graph is taken from Gordon & Gordon (1954).

males and females to its standard error is 0.8. The corresponding ratio for the depth index is 1.0, and that for the length of caudal peduncle index is 15.0. The length of caudal peduncle index is thus significantly greater in females than in males.

To eliminate the possible inclusion of immature specimens in comparing males with females, 50 females and 36 males, selected for their large size, were compared. Table 4 and Text-fig. 8 present the data obtained on these fish. No significant difference in length or depth index was found. As before, the females have a significantly larger length of caudal peduncle index. Since no significant difference was found in standard length, this result indicates a probable difference in the absolute length of the caudal peduncle. To test this hypothesis, the mean length of caudal peduncle for males was compared with that for females. The caudal

TEXT-FIG. 7. The measurements which were made on specimens of *Xiphophorus maculatus*. The horizontal line indicates the *standard length*. The vertical line indicates the *depth*. The diagonal line indicates the *length of caudal peduncle*. This figure represents a male.



peduncle was found to be significantly longer, 1.1 \pm .2 mm, in males than in females. An explanation of this difference, on the basis of differential growth in the sexes, will be suggested in the discussion.

Comparison of Fish with Different Macromelanophore Patterns

The results of comparing the measurements of fish having various macromelanophore patterns are presented in Table 2 for males and Table 3 for females, Text-figs. 9, 10 and 11. Significant differences were found (1) between the values for all males and those that are "+" (recessive) with respect to standard length; (2) between all males and Sr males with respect to depth indcx; and (3) between "+" males and Srmales with respect to standard length and depth index. No significant differences were found between any other groups, either males or females.

Comparison of Fish Collected at the Same Place on Different Days of the Same Week

The mean values of the standard length, depth index and length of caudal peduncle index for males obtained in four collections, '39-43a, b, c and d, made on March 4, 6, 7, 10, 1939, from the same pool, are presented in Table 5 and Text-fig. 12. The males collected on March 4 are significantly longer than those in each of the other three days' collections. The fish collected on March 6 are longer than those collected on March 10. The individuals in the collection of March 10 have a significantly greater depth index than those in the other collections; those in the collection of March 7 have a greater depth index than those in the collection of March 4. The fish collected March 10 have a lower length of caudal peduncle index than those in the other collections. No other significant differences were noted.

 TABLE 2. ANALYSIS OF DIFFERENCES BETWEEN MALES OF DIFFERING PHENOTYPES

 IN THE RÍO JAMAPA POPULATION OF Xiphophorus maculatus

Pattern	N		M	$\sigma_{\mathtt{M}}$	σ	$d/\sigma_d(\Sigma)$	d/0 _d (+)
 _+	292	S.L.	23.8	.218	3.723	5.4	
•		S.L./D.	2.36	.008	.137	.9	
		S.L./L.C.P.	2.10	.006	.102	1.2	
Sr	35	S.L.	25.6	.412	2.439	.5	2.8
		S.L./D.	2.25	.018	.107	4.3	4.6
		S.L./L.C.P.	2.12	.012	.071	.5	1.1
Sd	9	S.L.	24.8	.576	1.728	.5	.8
		S.L./D.	2.27	.028	.084	1.8	2.0
		S.L./L.C.P.	2.11	.026	.077	.0	.3
Sp	10	S.L.	25.8	.967	3.058	.5	1.7
-1		S.L./D.	2.40	.035	.111	1.1	1.0
		S.L./L.C.P.	2.15	.021	.066	1.4	1.5
All Males	346	S.L.	25.3	.177	3.287		5.4
		S.L./D.	2.35	.007	.130		.9
		S.L./L.C.P.	2.11	.006	.111		1.2

Explanation of symbols used above:

N = Number of specimens.

M = Mean.

 $\sigma =$ Standard deviation.

 $\sigma_{\rm M} =$ Standard error of mean.

S.L. = Standard Length.

S.L./D. = Standard Length divided by Depth.

S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.

 $d/\sigma_d(\Sigma)$ = The ratio of the difference between the mean for the pattern and the mean for all males to this difference's standard error.

 d/σ_d (+) = The corresponding ratio for + males instead of all males.

Comparison of Local Populations

A comparison between collections made at two nearby points in the Río Papaloapan is presented in Table 6. No significant differences were found.

Data for five collections from the Río Coatzacoalcos are given in Table 7 and Text-fig. 13. With regard to standard length, the only outstanding collection is GAW 32, which was significantly larger than the other populations collected. GAW 31, collected in the same general locality, has the smallest mean length and is significantly different from three of the other four collections examined. Most of the populations examined are significantly different from each other in the depth index. The only significant differences in the length of caudal peduncle index are between populations GAW 17, GAW 23 and population GAW 35.

The Río Tonalá sample was too small to permit comparisons within this river system.

Gordon (1947) found that the three populations in the Río Usumacinta system were genetically distinct with respect to the frequencies of melanophore pattern genes. Since the samples were too small to permit comparisons between individual stations, the samples were combined according to these groups and then compared. The data for the Río Usumacinta system are given in Table 8 and Text-fig. 14. In the comparisons of standard length, Group A (Laguna de Zotz) was significantly different from both Group B (Río de la Pasíon) and Group C (Laguna de Petenxil-Petén). Groups B and C were not significantly different. All three groups are significantly different in the depth index. Group A is significantly different in the length of the caudal peduncle index from Group B, but not from C. Group B is significantly different from C with respect to this index.

Comparison of the Seven River Systems

It will be found that the number of specimens from each river system shown in Table 9 differs from the total of the numbers for the local populations in that system. This is because samples too small to be used for local comparisons are included here. The comparative values are shown in Table 9 and Text-fig. 15.

1. With respect to standard length, the following observations may be made. The Río Jamapa specimens are significantly longer than all others. The Río Tonalá collection is not significantly different from either that of the Río Coatzacoalcos or that of the Río Usumacinta, its mean lying midway between their significantly differing means. The Río Grijalva specimens are not different from those of the

Pattern	N		М	σ _M	σ	$d/\sigma_d(\Sigma)$	d/σ₄(+)
+	435	S.L.	25.4	.179	3.723	.4	
		S.L./D.	2.32	.007	.146	.2	
		S.L./L.C.P.	2.23	.006	.125	.0	
Sr	35	S.L.	25.6	.489	2.893	.2	.3
		S.L./D.	2.34	.020	.118	.0	.8
		S.L./L.C.P.	2.24	.017	.100	.5	.5
Sd	1	S.L.	26.0	4		.1*	.1*
		S.L./D.	2.60			1.7*	1.9*
		S.L./L.C.P.	2.26			.3*	.2*
Sp	5	S.L.	27.6	1.431	3.205	1.2	1.3
		S.L./D.	2.37	.020	.045	.4	.8
		S.L./L.C.P.	2.21	.057	.128	.4	.3
All Fema	ales 476	S.L.	25.5	.169	3.686		.4
		S.L./D.	2.34	.007	.153		.2
		S.L./L.C.P.	2.23	.005	.109		.0

TABLE 3. ANALYSIS OF DIFFERENCES BETWEEN FEMALES OF DIFFERING PHENOTYPES IN THE RÍO JAMAPA POPULATION OF Xiphophorus maculatus

The symbols used above are explained in Table 2, except that $d/\sigma_d(\Sigma)$ in this table involves all females instead of all males.

* Only one Sd female was collected. Accordingly, the numbers given are ratios of the deviation of this female from the mean for the group with which it is being compared to the standard deviation for that group.

▲ ♂ ♀ 36 50	B & ç 36 50	C ở ç 36 50	D e ^a g 36 50
m. .	2.72 — 2.67 — 2.62 —	2.72 — 2.67 — 2.62 —	mn.
39.8	2.57 — 2.52 —	2.57 — 2.52 — 2.47 —	17.0
38.8	2.47	2.42	16.5 -
37.8	2.42 —	2.37	16.0
36.8 35.8	2.37	2.27	15.5
34.8	2.27	2.17	14.5 —
33.8	2.22 —	2.12 -	14.0
32.8 —	2.17	2.02 -	13.5 —
	2.12	1.97	13.0
29.8	2.02	1.92	12.0

TEXT-FIG. 8. Sexual dimorphism in the largest specimens of *Xiphophorus maculatus* in the Río Jamapa collection. The sections of the graph headed A, B, C and D refer to the standard length, depth index, length of caudal peduncle index and length of caudal peduncle, respectively. The graph is based on data on 36 males and 50 females; these data are given in Table 4. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean.

Río Coatzacoalcos and the Río Tonalá. The Belize River specimens are not significantly different from those of the Río Coatzacoalcos, Río Tonalá and Río Usumacinta. All other means showed significant differences.

2. In comparisons involving the depth index, the Río Tonalá population is found to be not significantly different from that of the Río Papaloapan or the Río Grijalva population from the Río Jamapa one. All other differences are significant. 3. The Río Grijalva specimens are not significantly different in the length of caudal peduncle index from the Belize River fish. All other differences are significant.

Correlation Between Standard Length and Length of Caudal Peduncle Index

Table 10 gives the correlation between standard length and the length of caudal peduncle index for each of the seven river populations. Only the Río Grijalva population shows a sig-

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TEXT-FIG. 9. Comparison of different macromelanophore patterns with respect to standard length in *Xiphophorus maculatus*. The data are for the Río Jamapa sample and are given in Tables 2 and 3. The symbol Σ designates the sum of all phenotypic groups. The numbers near the top are the sample sizes. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean.

nificant correlation; $z = -.35 \pm .15$. Of the seven correlation coefficients, five are negative. The mean value of the seven coefficients is $-.086 \pm .049$. The value of χ^2 for determining the possibility of all seven correlations arising simultaneously by chance is 11.40 (with seven degrees of freedom); the probability of a worse fit is about 0.12. The correlation coefficient for



TEXT-FIG. 10. Comparison of different macromelanophore patterns with respect to depth index in *Xiphophorus maculatus*. The data are for the Río Jamapa sample and are given in Tables 2 and 3. The symbol Σ designates the sum of all phenotypic groups. The numbers near the top are the same sizes. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean.

the Río Grijalva population differs significantly from those for the Río Tonalá and Río Usumacinta ones. No other significant differences are found among the correlation coefficients.

DISCUSSION

Sexual Dimorphism

Cohen (1946) found the mean length of *Xiphophorus maculatus* females significantly larger than that of males. This difference between his results and those obtained here may be due to the small number of specimens he used (seven of each sex) or to the fact that he used domesticated stock, raised under aquarium conditions. The difference in length accounts for the fact that he gives significantly different values of the depth index for males and females (3.61 and 2.82, respectively; see below).



TEXT-FIG. 11. Comparison of different macromelanophore patterns with respect to length of caudal peduncle index in *Xiphophorus maculatus*. The data are for the Río Jamapa sample and are given in Tables 2 and 3. The symbol Σ designates the sum of all phenotypic groups. The numbers near the top are the sample sizes. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean.

Bellamy (1922), on the other hand, obtained the same depth index for both males and females (2.7).

Our data indicate that males and females from the Río Jamapa do not differ in mean standard length nor in mean depth index. The caudal peduncle, however, is 1.1 mm (average) longer in males than in females. This is based upon fish that are 32 mm (average) in length.

The difference in length of the caudal peduncle between males and females may be attributed to fact that during morphogenesis of the male gonopodium, the anal fin moves to a more forward position (Gordon & Benzer,



TEXT-FIG. 12. Comparison of *Xiphophorus maculatus* taken from the same pool on different days of the same week. A, B, C and D indicate the collections made at station '39-43 on March 4, 6, 7 and 10, 1939, respectively. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean. The data on which this graph is based are given in Table 5.

1946). This realignment produces a relatively longer caudal peduncle in the male and a corresponding dissimilarity in the indices.

Since males differ from females with respect to the length of the caudal peduncle, it is probably better to use members of one sex in making comparisons between populations. In deciding which sex to use, it should be noted that males may be distinguished from females and immature fish by the andromorphic modifications of their anal fin. Females can, as a practical matter, be distinguished from immature fish only approximately on the basis of their size and their body contours. For this reason it is advantageous to use males in interpopulation comparisons. The use of males also eliminates the possibility of errors arising from the inclusion of temporarily deep-bodied gravid females. Accordingly, all comparisons between populations in the present analysis are based on males alone.



TEXT-FIG. 13. Comparison of five local populations of *Xiphophorus maculatus* in the Río Coatzacoalcos. The numbers near the top are station numbers. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean. The data on which this graph is based are given in Table 7.

Comparison of Fish of Different Macromelanophore Patterns

In comparing individuals of different phenotypes, males and females were considered separately. Thus the difficulties of considering both sexes at once are eliminated. The possibility that size differences between fish of different phenotypes may exist in one sex only is also not overlooked.

Males without a macromelanophore pattern (+) appear to be shorter than males marked

with a macromelanophore pattern. This difference, however, probably results from the inclusion of immature fish. The macromelanophore patterns develop in fish only as these fish reach a size comparable to that at which they mature. Accordingly, a macromelanophore pattern may not appear in a fish until after the modification of its anal fin has become sufficient for the fish to be classified as a male. Certain of the fish classified as "+" males would, therefore, have been expected to develop macromelanophore

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TEXT-FIG. 14. Comparison of three population groups of Xiphophorus maculatus in the Río Usumacinta. A, B and C refer to the Laguna de Zotz, Río de la Pasión and Laguna de Petenxil-Petén, respectively. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean. The data on which this graph is based are given in Table 8.

patterns if they had been permitted to live. For this reason the mean standard length of "+" males might be expected to be less than that of males with other phenotypes.

The separation of females from immature fish is based primarily on size. Fish large enough to be called females have, in general, developed their macromelanophore patterns. For this reason any difference in length between "+" females and females with macromelanophore patterns is minimized. The difference in depth index between Sr males and "+" males is probably a consequence of the fact that the Sr males are longer in mean than the "+" males, together with the tendency for longer fish to be relatively more deep (see below).

The absence of differences in size between the fish of different phenotypes permits one to combine all phenotypes in comparing populations.

One method by which the polymorphism, and



TEXT-FIG. 15. Comparison of seven river populations of *Xiphophorus maculatus*. J, P, C, T, G, U and B refer to the Río Jamapa, Río Papaloapan, Río Coatzacoalcos, Río Tonala, Río Grijalva, Río Usumacinta and Belize River, respectively. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean. The data on which this graph is based are given in Table 9.

in particular the diversity of macromelanophore pattern, of the platyfish might be maintained is through selection. The data indicate that any selection which may be present operates in a manner which neither causes, nor is caused by, substantial size differences. Since only a weak selection pressure is sufficient to maintain polymorphism, differences in size too small to be detected by the present analysis may occur and yet have significance with regard to selection. On the other hand, since selection is not necessarily associated with size differences, the data here considered can at best have merely negative significance with regard to determining whether selection is present.

Direct Comparison of Natural Populations with Respect to Size and Shape

The difficulty in comparing natural populations of platyfish by means of measurements is not one of finding characteristics which differ from population to population. The question is rather how to find dimensions and indices that reflect permanent characteristics of the populations.

The '39-43 collection is valuable for determining the validity of using any particular dimensions and indices as means of distinguishing populations. Statistically significant differences are found between the fish taken on different days with respect to both the depth index and the length of caudal peduncle index, as well as with respect to length. Since all fish taken at station '39-43 represent the same population, it is clear that differences found in either of these indices between two samples do not reflect genetic dif-

TABLE 4. ANALYSIS OF DIFFERENCES BETWEEN THE LARGEST MALES AND FEMALES OF A COLLECTION OF *Xiphophorus maculatus* taken in the Río Jamapa

Sex	N		M	$\sigma_{\rm M}$	σ	d/od
ð	36	S.L.	31.2	.195	1.170	.2
		S.L./D.	2.31	.019	.114	.3
		S.L./L.C.P.	2.10	.015	.090	9.6
		L.C.P.	14.9	.164	.984	6.4
ę	50	S.L.	32.2	.325	2.298	
		S.L./D.	2.30	.020	.141	
		S.L./L.C.P.	2.33	.017	.120	
		L.C.P.	13.8	.142	1.004	

Explanation of symbols used above: N = Number of specimens.

M = Mean.

 $\sigma =$ Standard deviation.

 $\sigma_{\rm M} = {\rm Standard\ error\ of\ mean.}$

S.L. = Standard Length.

S.L./D. = Standard Length divided by Depth.

S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.

The column headed d/σ_a gives the ratio of the difference between the mean for females and that for males to its standard error. ferences within the populations from which the samples were drawn. The indices for a population apparently may change appreciably within a week. Accordingly, if the indices can be used at all to distinguish populations, they must be used with caution.

The variations in length and in the indices from day to day in the population at station '39-43, whatever their causes, must be considered in comparing populations. Nevertheless, an attempt was made to explain the variation; a knowledge of the causes of the differences might make it possible to devise effective methods of comparing populations. The mean standard length of the fish collected decreased from day to day. Gordon (1947) said that predatory birds and fishes eliminated relatively more of the larger platyfish between the four collections. The pool that held the platyfish was shallow and the water was rapidly evaporating. On the last collection from the pool all fish species were represented mainly by their young.

The differences found among the various days' collections would be expected if there were negative correlation between the depth index and the standard length, and between the length of caudal peduncle index and the standard length. The appropriate correlation coefficients were therefore examined. In the case of the depth index vs. standard length correlation, the 75 males from the Río Usumacinta give a value of the correlation coefficient $z = -.51 \pm .12$. Seventy-five males from the Río Tonalá also

 TABLE 5. BIOMETRIC COMPARISON¹ OF FOUR COLLECTIONS OF Xiphophorus maculatus

 MADE FROM THE SAME POOL² IN THE RÍO PAPALOAPAN WITHIN A WEEK

Day of Collection	Number Specime	of ns	Mean	Standard Deviation	Ratio of D Standard March 6	Difference in Error as Com March 7	Mean to Its apared with March 10
March 4, 1939	473	S.L. S.L./D. S.L./L.C.P.	21.3 ± 1.75 $2.35 \pm .006$ $2.32 \pm .008$	3.82 .136 .163	3.4 2.0 4.1	2.5 2.5 6.2	12.1 10.0 5.5
March 6, 1939	48	S.L. S.L./D. S.L./L.C.P.	19.4±.293 2.39±.017 2.42±.024	2.08 .117 .166		1.5 .0 .7	2.1 3.2 1.9
March 7, 1939	85	S.L. S.L./D. S.L./L.C.P.	$20.2 \pm .361$ $2.39 \pm .014$ $2.44 \pm .018$	3.34 .129 .167			.5 2.6 1.7
March 10, 1939	404	S.L. S.L./D. S.L./L.C.P.	$18.6 \pm .122$ 2.44 ± .007 2.38 ± .005	2.53 .130 .156			

¹ The following are used in the comparison:

S.L. = Standard Length.

S.L./D. = Standard Length divided by Depth.

S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.

² Station number '39-43.

Collection Number	Number of Specimens		Mean	Standard Deviation	Ratio of Difference in Mean to Its Standard Error as Compared with '39-43
'3 9-38	321	S.L. S.L./D. S.L./L.C.P.	$20.1 \pm .254 \\ 2.39 \pm .008 \\ 2.34 \pm .009$	3.51 .149 .153	.5 0.0 1.4
'39-43	1010	S.L. S.L./D. S.L./L.C.P.	$20.0 \pm .110$ $2.39 \pm .005$ $2.36 \pm .005$	3.51 .174 .166	

 TABLE 6. BIOMETRIC COMPARISON¹ OF TWO LOCAL POPULATIONS OF Xiphophorus maculatus

 FROM THE RÍO PAPALOAPAN

¹ The following are used in the comparison:

S.L. = Standard Length.

S.L./D. = Standard Length divided by Depth.

S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.

give $z = -.51 \pm .12$. Scatter diagrams indicated that similar values would be obtained for the other populations. In view of the good correlation in the Río Usumacinta and Río Tonalá collections and the scatter diagrams, it seemed unnecessary to make any further calculations. It may be concluded that as a fish grows larger, it grows relatively deeper.

Whether there is any correlation between the length of caudal peduncle and standard length

is not clear. Since no definite results with preliminary methods were obtained, correlation coefficients were calculated for all seven river populations (Table 10). The coefficients obtained differ significantly among themselves. It is possible that the correlation coefficient obtained from any given sample depends on the mean length and range in length of the fish in the sample. Thus the differences in correlation coefficients among the samples may

Ratio of Difference in Mean to Its Standard Collection Number of Standard Error as Compared with Number Specimens Mean Deviation GAW 17 **GAW 32 GAW 23 GAW 31** $21.6 \pm .799$ S.L. 4.45 3.2 .2 6.1 2.5 **GAW 35** 31 S.L./D. $2.36 \pm .026$.174 5.0 1.1 5.9 4.0 S.L./L.C.P. $2.21 \pm .018$.103 3.6 2.7 3.0 1.7 S.L. $19.8 \pm .152$ 2.72 4.4 15.8 1.8 **GAW 17** 321 S.L./D. $2.50 \pm .008$.321 1.9 10.0 3.1 S.L./L.C.P. $2.12 \pm .002$.135 .4 0.0 1.9 S.L. $21.8 \pm .373$ 2.36 9.0 3.9 **GAW 23** 40 S.L./D. $2.44 \pm .059$.373 3.1 2.0S.L./L.C.P. $2.13 \pm .022$.137 .3 1.2 S.L. $27.8 \pm .586$ 2.99 9.4 **GAW 32** 26 S.L./D. $2.20 \pm .029$.150 9.7 S.L./L.C.P. $2.12 \pm .024$.121 1.5 S.L. $18.8 \pm .734$ 3.95 **GAW 31** 29 S.L./D. $2.59 \pm .028$.153 S.L./L.C.P. $2.17 \pm .023$.125

 TABLE 7. BIOMETRIC COMPARISON¹ OF FIVE LOCAL POPULATIONS OF Xiphophorus maculatus

 FROM THE RÍO COATZACOALCOS

¹ The following are used in the comparison:

S.L. = Standard Length.

S.L./D. = Standard Length divided by Depth.

S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.

not reflect any essential differences among the populations. Combining all the data either by using χ^2 or by taking a mean value gives a probability of about 0.1 of obtaining as much or more correlation by chance. In view of this low, although not significantly low, probability and the significant correlation obtained from the Río Grijalva sample, it may well be that, at least in some populations, there is negative correlation between the standard length and the length of the caudal peduncle index.

The local populations within each river system and the seven river systems may be compared by the means of the indices of the length of caudal peduncle. As shown above, the correlation between it and the standard length, if there is any at all, is small. It may also be noted that collections GAW 31 and GAW 32, which were made at nearby points in the Río Coatzacoalcos, do not differ significantly for this index, although they show a great difference (9 mm) in standard length. Again, in the Río Usumacinta region, the group collections from the Laguna de Zotz and those from the Laguna de Petenxil-Petén, which would be expected to resemble each other to some degree because of their geographical proximity, show a non-significant difference only for this index.

On the other hand, the March 4, 1939, collection from the Río Papaloapan differs significantly with respect to this index from the other three collections made within a week from the same population. It may be noted that Gordon & Benzer (1945) found that the differences which exist in vertebral counts between the seven species of xiphophorin fishes occur in the region of the caudal peduncle. The length of caudal peduncle index is probably better than the standard length or depth index for comparing populations. Its validity for this purpose is nevertheless doubtful; if it can be used at all, it must be used with considerable caution.

With these various limitations in mind, the following comparisons of local groups may be presented.

1. There are no significant differences between two representative Río Papaloapan populations.

2. In the Río Coatzacoalcos populations, considering only the length of caudal peduncle index, the only significant differences occur between collection GAW 35 and collections GAW 17, GAW 23 and GAW 32. These differences may reflect geographical factors, such as distance or altitude. GAW 35 was taken at a place somewhat removed from those where the other three collections were made (Text-fig. 4). It should be noted that none of the differences is very great, however.

3. In the populations from the upper Río Usumacinta region, the Río de la Pasión group is significantly different from the Laguna de Zotz and Laguna de Petenxil-Petén groups with respect to the length of caudal peduncle index, but the latter two groups do not differ significantly from each other. This may be accounted for by the fact that the Laguna de Zotz and the Laguna de Petenxil-Petén are located more closely to each other.

With similar limitations upon significance of the differences found, the following comparisons between the seven river populations taken as

					Ratio of Differ Standard Error	ence in Mean to Its as Compared with
Location	Number Specimer	of ns	Mean	Standard Deviation	Río de la Pasión	Laguna de Petenxil-Petén
Laguna de Zotz	94	S.L. S.L./D. S.L./L.C.P.	$19.9 \pm .305$ 2.74 ± .013 2.36 ± .016	2.96 .130 .152	8.7 12.6 6.5	7.3 7.4 1.7
Río de la Pasión	51	S.L. S.L./D. S.L./L.C.P.	24.8±.507 2.27±.021 2.20±.017	3.62 .148 .120		.7 2.7 3.2
Laguna de Petenxil-Pet	én 25	S.L. S.L./D. S.L./L.C.P.	$25.5 \pm .931$ $2.36 \pm .021$ $2.30 \pm .028$	4.65 .106 .139		

TABLE 8. BIOMETRIC COMPARISON¹ OF THREE LOCAL POPULATIONS OF Xiphophorus maculatus FROM THE RÍO USUMACINTA SYSTEM

¹ The following are used in the comparison:

S.L. = Standard Length. S.L./D. = Standard Length divided by Depth.

S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.

maculatus
Xiphophorus
OF
POPULATIONS
RIVER
SEVEN
OF
COMPARISON ¹
BIOMETRIC
9.
TABLE

					Ratic	o of Difference in	Mean to Its :	Standard Erro	r as Compared w	ith
N River S _l	umber o	of Is	Mean	Standard Deviation	Río Papaloapan	Río Coatzacoalcos	Río Tonalá	Río Grijalva	Río Usumacinta	Belize River
Jamapa	346	S.L. S.L./D. S.L./L.C.P.	25.3±.177 2.35±.007 2.11±.006	3.29 .130 .111	65.5 4.4 28.0	18.2 10.3 39.6	9.9 2.9 21.7	9.4 1.4 7.4	7.5 13.7 18.9	6.4 25.6 7.5
Papaloapan	1331	S.L. S.L./D. S.L./L.C.P.	20.0±.030 2.39±.004 2.36±.004	1.11 .155 .156		7.1 8.3 27.8	10.9 .5 6.2	4.0 11.3 5.8	16.7 13.4 2.4	10.7 23.5 5.2
Coatzacoalcos	485	S.L. S.L./D. S.L./L.C.P.	20.8±.167 2.46±.008 2.62±.010	3.68 .165 .222			2.0 3.0 5.2	.2 3.3 12.3	5.1 6.6 16.0	2.1 18.2 11.7
Tonalá	81	S.L. S.L./D. S.L./L.C.P.	$21.7\pm.389$ $2.40\pm.018$ $2.48\pm.032$	3.50 .162 .292				1.7 17.8 5.9	1.5 5.8 5.4	.4 14.9 5.4
Grijalva	50	S.L. S.L./D. S.L./L.C.P.	20.7±.400 2.64±.017 2.23±.012	2.80 .116 .082					2.6 8.9 4.5	2.0 7.7 .4
Usumacinta	176	S.L. S.L./D. S.L./L.C.P.	22.6±.362 2.57±.018 2.33±.011	4.81 .238 .151						3.3 8.8 8.8
Belize	48	S.L. S.L./D. S.L./L.C.P.	22.0±.507 2.94±.036 2.24±.017	3.52 .249 .120						
¹ The followir S.L. = Standa S.L./D. = Sta S.L./L.C.P. =	ng are u urd Leng ndard L Standa	the comparation of the comparati	arison: Depth. 1 by Length of	f Caudal Pedu	incle.					

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wholes may be considered. The differences in standard length between the river populations show no clear linear geographical trend. The depth index increases from north to south in an orderly manner; the trend is perfect except for two cases in which neighboring populations are reversed. Thus, platyfish become relatively deeper from south to north. The length of caudal peduncle index seems to increase southward from the Río Jamapa to the Río Coatzacoalcos and then to decrease from the Río Coatzacoalcos to the Belize River.

Other Possible Methods of Comparing Populations

Various procedures have been considered in comparing the populations to avoid the difficulties and uncertainties mentioned above. For example, consideration was given to the possibility that only fish of a certain length, i.e., range in length, be used in comparing populations. All specimens not of the length chosen would be ignored. One of the disadvantages of this method is the difficulty of obtaining a sufficient number of specimens of the same length. Even if enough specimens of one size are obtained, there would be an objection to the validity of the procedure. The selection of certain specimens from each population might result in specimens which were among the largest in their population being compared with others which were among the smallest in theirs. This procedure thus might produce differences in the indices that do not reflect the real differences in the populations.

Another procedure for comparing populations that was considered is to examine the rectilinear regression coefficients. If the relation between a given dimension and the fish's length is linear, the regression coefficients would be unaffected by the lengths of the specimens in a sample being studied. In that case, a comparison of regression coefficients is a valid method of comparing populations. There is, however, no reason

 TABLE
 10.
 CORRELATION
 Between
 Standard

 LENGTH AND STANDARD LENGTH DIVIDED BY LENGTH
 OF
 CAUDAL
 PEDUNCLE
 IN
 Xiphophorus
 maculatus

	r	z	σz	z/σ _z
Río Jamapa	141		.12	1.2
Río Papaloapan	159	—.16	.12	1.3
Río Coatzacoalcos	066	07	.12	.6
Río Tonalá	+.144	+.15	.12	1.0
Río Grijalva	335	35	.15	2.3
Río Usumacinta	+.044	+.04	.12	.4
Belize River	090	09	.15	.6

to suppose that the requisite linearity exists. If a dimension is not linearly related to length, the regression of that dimension on length will be affected by the lengths of the specimens in the sample, i.e., two samples of different mean length from the same population may have different regression coefficients. Accordingly, the regression coefficients are not necessarily more reliable than dimensions and indices in comparing populations. In this connection, Schaefer (1952) used the study of regression lines to compare yellowfin tunas, Neothunnus macropterus, from Hawaiian waters with those from the American West Coast. He noted, however, that the regression analysis was "beset with difficulties" and found that only because the magnitude of the differences between populations was sufficient, was he able to obtain results.

General Uniformity of the Male Platyfish in Each of the Seven River Systems

The male platyfish in each of the seven river populations do not differ in mean standard length from 22.7 mm by more than 12%; in mean depth index from 2.65 mm by more than 11%; or in mean length of caudal peduncle index from 2.37 mm by more than 11%. These percentages are equivalent to about 2 mm in length and about 1 mm in depth or length of caudal peduncle. It may be noted that the platyfish is also uniform in size with respect to comparisons between fish of different sex and between those of different macromelanophore pattern. The decrease in relative depth from north to south very likely reflects a real difference between the populations. Aside from this trend, the data indicate that it is the uniformity, rather than the diversity between isolated populations that is significant.

Although platyfish are uniform as to size and shape, the seven river populations can all be recognized as distinct on the basis of the frequencies of their melanic patterns (Gordon & Gordon, 1954). Thus it may be said that the meristic criteria used in this study for distinguishing populations of platyfish are not as precise as those based upon their inherited color patterns.

SUMMARY

The platyfish, *Xiphophorus maculatus*, has been collected from seven major rivers which empty into the Atlantic Ocean from Mexico to British Honduras. Measurements were made on a total of 2,993 adult platyfish from these seven river systems with reference to their standard length and two body indices, namely, the standard length divided by the depth of body and the standard length divided by the length of caudal peduncle. Males and females were compared separately, since it was found that the length of caudal peduncle was slightly longer in males than in females.

No significant differences were found in the body measurements and proportions between fish with various inherited macromelanophore patterns, or fish without these patterns. Since polymorphism in platyfish is probably maintained by a weak selection pressure, differences in size too small to be detected by the present analysis may occur and yet have significance with regard to selection. On the other hand, since selection is not necessarily associated with size differences, the data here considered can at best have only negative significance with regard to determining whether selection is present.

Statistically significant differences were found in standard length and in the body indices among four collections of adult male platyfish taken from the same pool on four different days within one week. In view of this great variability, the measurements employed seem inadequate for the purpose of differentiating the seven populations of platyfish. However, the mean relative depth of the platyfish appears to decrease progressively from north to south. The other measurements do not follow this trend.

The seven isolated populations of platyfish are essentially similar with regard to their size and body proportions. Because of this, the meristic criteria used in this study in an attempt to distinguish the seven populations are not as precise as those based upon the frequencies of their inherited color patterns.

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References

BELLAMY, A. W.

- 1922. Breeding experiments with the viviparous teleosts, Xiphophorus lielleri and Platypoecilus maculatus (Günth.). Anat. Rec., 23: 98-99. (Abstract).
- Cohen, Herman
 - 1946. Effects of sex hormones on the development of the platyfish, *Platypoecilus maculatus*. Zoologica, 31: 121-128. Co., Inc., New York, 414 pp.

- DICE, L. R. & H. J. LERAAS
 - 1936. A graphic method for comparing several sets of measurements. Cont. Lab. Vert. Genetics, Univ. Michigan, No. 3: 1-3.
- GORDON, HUGH & MYRON GORDON
 - 1950. Colour patterns and gene frequencies in natural populations of a platyfish. Heredity, 4: 61-73.
 - 1954. Maintenance of polymorphism by potentially injurious genes in seven natural populations of the platyfish, *Xiphophorus maculatus*. Jour. Genetics (in press).

GORDON, MYRON

- 1947. Speciation in fishes. Distribution in time and space of seven dominant multiple alleles in *Platypoecilus maculatus*. Advances in Genetics, 1: 95-132.
- 1948. Effects of five primary genes on the site of melanomas in fishes and the influence of two color genes on their pigmentation. Biol. of Melanomas, Spec. Publ., N. Y. Acad. Sci., 4: 216-268.
- 1950. The origin of modifying genes that influence the normal and atypical growth of pigment cells in fishes. Zoologica, 35: 19-20. (Abstract).
- 1951a. The variable expressivity of a pigment cell gene from zero effect to melanotic tumor induction. Cancer Research, 11: 676-686.
- 1951b. Genetics and correlated studies of normal and atypical pigment cell growth. Growth, Symposium, 10: 153-219.

GORDON, MYRON & PAUL BENZER

- 1945. Sexual dimorphism in the skeletal elements of the gonopodial suspensoria in xiphophorin fishes. Zoologica, 30: 57-72.
- Hoy, H. E.
 - 1943. Physiographic diagram of Mexico. Papers, Michigan Acad. Sci., 28: 441-443.
- HUBBS, C. L. & HENRY VAN DER SCHALIE
 - 1937. Map of El Petén, Guatemala, and bounding regions of British Honduras and Mexico (Chiapas, Tabasco, and Campeche). Univ. Michigan, Ann Arbor.
- PARR, A. E.
 - 1949. An approximate formula for stating taxonomically significant proportions of fishes with reference to growth changes. Copeia, 1949: 47-55.

SCHAEFER, M. B.

1952. Comparison of yellowfin tuna of Hawaiian waters and of the American west coast. U. S. Fish and Wildlife Service, Fish. Bull. No. 72, 52: 353-373.

SIMPSON, GEORGE G. & ANNE ROE

1939. Quantitative Zoology. McGraw-Hill Book